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Predicting bloom dates by temperature mediated kinetics of carbohydrate metabolism in deciduous trees

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ABSTRACT

Trees in seasonal climates gauge winter progression to assure vital and productive blooming. However, how dormant plants asses environmental conditions remains obscure. We postulated that it involves the energetic reserves required for bloom, and therefore studied winter carbohydrate metabolism in deciduous trees.

We quantified non-structural carbohydrates throughout winter in almond, peach, and pistachio trees in California and Israel and characterized winter metabolism. We constructed a carbohydrate-temperature (C–T) model that projects changes in starch and soluble carbohydrate concentrations by temperature mediated kinetics. Then, we tested the C–T model projections of bloom times by 20 years of temperature and phenology records from California.

The C–T model attributes winter carbohydrate regulation in dormant trees to continuous updates of metabolic pathways. The model projects a surge in starch synthesis at the end of winter, and critically low concentrations of soluble carbohydrates, that trigger bloom. This is supported by field measurements of starch accumulation at the end of winter (~50 mg g^{-1} DW in almonds) that preceded bloom by ~10 days.

The C–T model provides a physiological framework for bloom forecasts in deciduous orchards. It integrates contrasting notions of chill and heat and elucidates why abnormal winter temperatures may compromise bloom in deciduous orchards.

1. Introduction

Vital and timely bloom is critical for the survival of many perennial species that rely on cross-pollination. While bloom time varies from year to year, in any specific year deciduous tree species bloom in synchrony across vast landscapes, e.g. cherry in Japan (Miller-Rushing et al., 2007), pistachios in California (Pope et al., 2014), and almonds in Spain (Egea et al., 2005). Evidently, once triggered, bud burst and flower development are under the plant's autonomous genetic control [MADS-box genes; (Becker and Theißen, 2003)]. Yet, while dormancy break was extensively surveyed at the bud level (Lloret et al., 2018; Vimont et al., 2018), whole-tree physiological processes that relate bloom to environmental cues remain obscure. It is assumed that winter temperatures and photoperiod are the main environmental cues affecting winter phenology (Maurya and Bhalerao, 2017). However, in the productive Mediterranean and warm-temperate climates, temperature is the prominent cue for trees to determine winter progression and spring arrival (Guo et al., 2014). Thus, we tend to associate recently observed changes in blooming patterns and reproductive capacity in orchards and natural ecosystems to global climatic shifts in winter temperatures (Schleip et al., 2008). However, we are still missing a mechanistic link between temperature and spring phenology (Richardson et al., 2013) that could guide farming practices, breeding programs, and forest management in mitigating future impacts of climate change (Campoy et al., 2011).

Presumably, deciduous trees gauge winter progression by tracking temperature changes. Therefore, current empirical models use fall and winter temperatures to assess tree readiness for bloom (Luedeling and Brown, 2011). Traditionally, these models focused on chill and logged the hours trees spent below a specific temperature [commonly 7.2 °C,

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(Luedeling et al., 2009a)]. Later approaches considered multiple temperature thresholds in which tree inclination to bloom either increased in lower temperatures or decreased in warmer times [e.g. the Utah model, (Richardson et al., 1974)]. Finally, there is the Dynamic Model approach (Erez et al., 1990), which is more sophisticated as it tracks an intermediate component (a hypothetical physiological attribute) by the temperature kinetics of its formation and decay. Then, if this degradable component reaches an arbitrary threshold, the Dynamic Model counts an irreversible chill portion. These models, and their derivatives, aim to determine if winters are sufficiently cold for effective bloom of various tree crops. Yet, to account for the actual bloom time, these models are coupled with an additional empirical 'heat' model that accounts for the time trees spend in favorable growth conditions [i.e. growth degree hours (GDH), (Hunter and Lechowicz, 2008)], after exposure to chilling temperatures (Luedeling et al., 2009b). An implication of the combined chill-heat approach is that the more chill time trees accumulate, the less heat time they later require to break dormancy, and vice versa (Pope et al., 2014). Yet, with field observations often suggesting that chill accumulation is reversed by unusual warm days in winter (Erez et al., 2008), some of these empirical models malfunction in abnormal or warm weather conditions (Luedeling, 2012a). It renders them difficult to use, and they could become even less reliable considering recent climate changes (Chuine, 2000; Chuine et al., 2016). Nevertheless, these models could improve, if the biological mechanisms that enable trees to track winter progression by 'logging' temperatures (cold or hot) from senescence until bloom are unidentified.

There is mounting evidence that non-structural carbohydrate (NSC) metabolism plays a role in breaking dormancy and could provide mechanistic grounds to model winter chill accumulation (Fernandez et al., 2019; Tixier et al., 2019). No doubt, cell epigenetic modifications play a big role in driving spring phenology (Amasino, 2004). This resonates with climate modelling because plants often require chill before they silence their inflorescence repressors and promote bloom (Sung and Amasino, 2004). Moreover, the intercellular signaling pathways associated with dormancy are also sensitive to temperature changes (Rinne et al., 2011). Similarly, high soluble carbohydrates (SC) concentrations (González-Rossia et al., 2008), and genes associated with NSC metabolism (Anderson et al., 2005), already have been attributed to chill and to shifts from endo-dormancy to eco-dormancy (Charrier et al., 2018). Additionally, some controls of bud burst are associated with hexose (Fernandez et al., 2019), although bloom could be indifferent to the concentrations of other sugars (Maurel et al., 2004). Generally, tree NSC management, driven by photosynthesis and growth during spring and summer, depends on residual metabolism during winter, and is fueled solely by starch (ST) degradation during dormancy (Dietze et al., 2014). Turning starch reserves to soluble carbohydrates that are easily transported and energetically available requires little energy and plants can accelerate it according to their respiratory needs (Sperling et al., 2015), or in response to abrupt changes in their environment [e.g. frost (Sperling et al., 2017a)]. These SC concentrations protect plants from starvation and sustain osmotic balances (Granot et al., 2013). SC concentrations are therefore constantly recharged from starch reserves and hence dormant trees often deplete their canopy ST storages by the end of winter (Ashworth, 1993). However, SC concentration and composition in plant tissues is regulated (Lalonde et al., 1999) and probably won't exceed a metabolically permissive concentration (SCm) during winter, e.g. 50 to 100 mg g^{-1} DW in dogwood or cherry (Ashworth, 1993; Loescher et al., 1990). It implies that trees sense their local sugar status [e.g. intracellular and apoplastic soluble sugar concentrations (Tadege et al., 1998)], and that they constantly adjust starch to SC catabolism and SC to starch synthesis (Witt and Sauter, 1994; Yoshioka et al., 1988). Subsequently, as spring growth takes its energetic toll, SC concentrations finally drop (Ashworth, 1993), although ST concentrations often surge surprisingly for a short period (Ito et al., 2012; Kaufmann and Blanke, 2017; Sauter and van Cleve, 1994). Therefore, a mechanistic explanation for the ability of trees to account for winter progression should consider the interaction between winter temperatures and NSC metabolism.

A key link between temperature and NSC metabolism in stem parenchyma cells is the temperature kinetics of SC and ST interconversion metabolic pathways, which are markedly different. ST synthesis is highly sensitive to temperature changes while ST degradation is not (Pollock and Lloyd, 1987). Hence, assuming that ST synthesis and degradation rates are equal at a specific temperature (Te), higher temperatures (T > Te) would promote ST accumulation over SC, and lower temperatures would promote ST degradation to SC (Zwieniecki et al., 2015). Effectively, a prolonged winter chill could lead to excessive SC accumulation in plants if it isn't negated by either increased respiration (Sperling et al., 2015) or adjustments to the ST synthase and degradation pathways. Such adjustments could result in ST accumulation as is the case of reproductive tissues in sweet cherry (Fadón et al., 2018). In this case, metabolic adjustments (genetic expression changes to the frequency of the reactions) to the cold could be the key to temperature 'logging' in dormant trees, and sensing SC concentrations could be the biological cue to spring arrival. Interestingly, SC synthesis being insensitive to temperature changes may result in plants being able to quickly respond to excessive SC (by synthesizing ST) but slow at dealing with heat driven SC deficiencies (due to accelerated respiration and starch synthase).

We set out to underline a physiological framework to model dormant tree winter temperature requirements, and link it to bloom time. We hypothesized that ***if dormant trees regulate SC concentrations during winter by updating ST synthesis in the cold, and ST degradation is less sensitive to temperature changes, the warmer temperatures in spring would lower SC, and could trigger bloom***. To test this hypothesis, we developed a novel mechanistic carbohydrate-temperature (C–T) model that projects NSC concentrations by hypothetical adjustments to the ST synthesis and degradation pathways by winter temperatures. To parametrize the C–T model we studied temporal dynamics of starch and SC concentrations in dormant almond [*Prunus dulcis* (Mill.) D.A. Webb] trees in the Central Valley of California, USA. We worked on twigs, rather than buds, as they better represent the NSC reserves available for spring growth (Tixier et al., 2018), and we studied in winter time before buds burst and take over carbohydrate metabolism. To test the model's predictions concerning NSC concentrations, we studied starch and SC of almond (*Prunus dulcis*), peach [*Prunus persica* (L.) Batsch], and pistachio [*Pistacia vera* (L.)] orchards in California and Israel. Finally, to test the model's predictions of bloom time, we processed historical datasets of hourly temperatures and phenology at three sites in the Central Valley of California.

2. Methods

2.1. Wood sampling and lab analysis

The model was parameterized by extensive sampling of ˜100 samples a month (1,100 in total) across California's almond production regions and conducting lab analyses for NSC content. This was a 'Citizen Scientist' initiative that worked with Californian farmers to send dry twigs (1st year of growth) by mail, starting July 2016 through May 2017 (see the 'Carbohydrate Observatory' project - https://psfaculty.plantsciences.ucdavis.edu/ plantsciences_faculty/zwieniecki/CR/cr.html). The farmers' work was complemented by sampling almond, pistachio, and peach trees (10 each) at research sites in California (UC Davis) and Israel (Gilat, ARO) every 14 days during winter. Lab NSC analysis followed an updated version of the anthrone method which is elaborated in the supplementary information (S4) and is further detailed and updated in our online portfolio (DOI: 10.13140/RG.2.2.25202.76480). Generally, NSC concentrations in dry weight (DW) of almond stem tissue are reported. However, the ratio of ST to SC (ST:SC) is used when almonds are compared to peach and pistachio

trees for normalization. Field sampling was mostly done by the farmers, which couldn't coordinate the work, and therefore samples taken within 3 days were aggregated and their NSC concentrations were averaged for this report (average number of repetitions was 5.3).

2.1.1. Phenology and meteorological data

Almond phenology was tracked at UC Davis during 2016–2017, combined with temperature records for Merced, CA (adjacent to the majority of the Carbohydrate Observatory sites), to parameterize the C–T model. In addition, the model and its capacity to project almond blooming time was tested using a phenology data set from the University of California Almond Regional Variety Trials from 1983 to 2011 that consisted of yearly dates for 10% bloom in three key almond cultivation sites in California (Durham, Manteca, and Shafter) (Pope et al., 2014).

Hourly air temperature data from the Californian Irrigation Management Information System (CIMIS, www.cimis.water.ca.gov) were used for four almond farming sites in California – Davis (38.54, −121.80), Shafter (35.45, −119.23), Durham (39.69, −121.83), and Manteca (37.83, −121.22). Data for the years 1982–2017 were used (if available), days with missing data were excluded (in total, only 11 days were missing for all the presented years and sites), and statistics were computed in the R (version 3.5.2) RStudio environment (version 1.1.463). Days between October 1st [the best fit to the time of physiological senescence in chill models for California's climate (Jarvis-Shean et al., 2011)] and May 1st (a time markedly after blooming) were counted as days past senescence (DpS), for all later computations and discussions. Overall, winter temperatures in these sites was similar - it averaged at 10 °C and 91% of the records were of temperatures between 0 °C and 20 °C. There were however annual minimums of −9 °C and occasional heat waves of 38 °C during late autumn (especially in October and early November).

3. Theory and calculations

3.1. Carbohydrate-Temperature (C–T) model development

We propose a mechanistic relationship between carbohydrates and ambient temperature, based on the activity of starch synthase and degradation enzymes, which elucidates the impacts of chill and heat on dormant trees. A simple, reversible, bi-enzyme sub-model was followed in which starch (ST) reacts with degrading enzymes (Ed) and turns to SC, while SC is lost to respiration (R) or reacts with synthesis enzymes (Es) that turn it back to starch (Menten and Michaelis, 1913):

$$\text{Model 1: } ST + Ed \underset{Ks}{\overset{Kd}{\rightleftarrows}} \boldsymbol{SC} + Es \xrightarrow{R} CO_2$$

The output of the model is hourly SC concentrations (highlighted by bold letters in **Mod. 1**).

For this bi-enzyme process to depend on temperature, the temperature kinetics of its independent enzymes need to differ (Kotov et al., 2007). The temperature kinetics of Ks and Kd rates are exponentially characterized by the frequency factors (As and Ad) and energy of activation (Bs and Bd) of their metabolic pathways (Chen and Tian, 2005):

$$K(T) = A \bullet \exp(BT) \quad (1)$$

This is consistent with the Arrhenius equation where the energy of activation is described by the change in activity at a 10 °C difference (Q_{10}) which biologically relates to starch synthesis ($Q_{10}s = 3$) and starch degradation ($Q_{10}d = 1.8$) (Pollock and Lloyd, 1987):

$$B = \boldsymbol{log}(Q_{10})/10 \quad (2)$$

The immediate implication of these temperature mediated starch kinetics of degradation and synthesis is that their activity rates equal at a specific temperature (Te; Fig. 1a):

$$Te = \frac{\log(As/Ad)}{Bd - Bs} \quad (3)$$

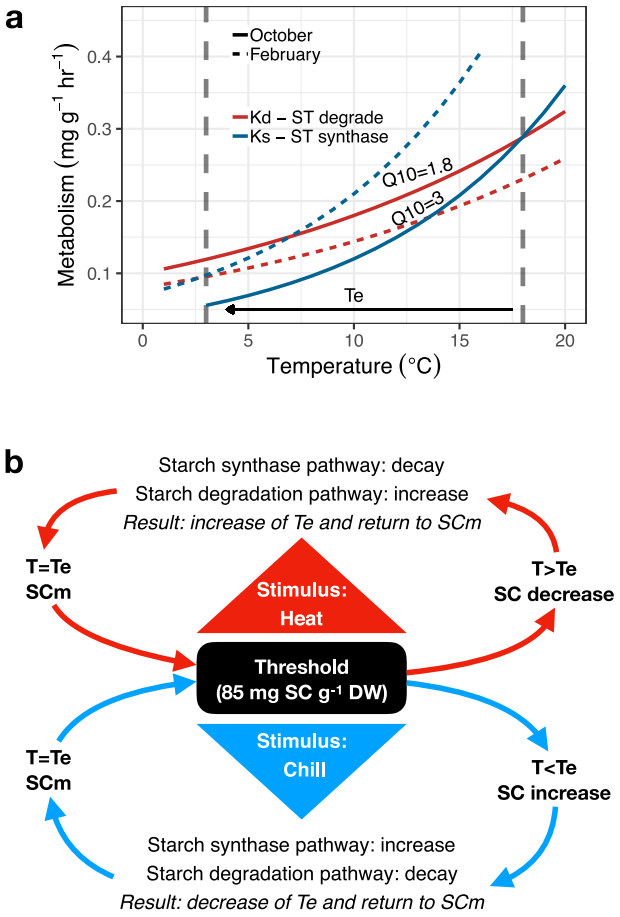

Fig. 1. A schematic overview of the C–T model. (a) Hypothetic starch (ST) synthesis (Ks, blue lines) and degradation (Kd, red) at senescence (October, continuous lines) and in bloom (February, dashed lines) rates for temperatures between 0 °C and 30 °C. The point where two exponential curves converge denotes the temperature of carbohydrate equilibrium (Te) for the two seasons. **(b)** A diagram to illustrate how chill (blue frames) and heat (red frames) stimuli activate metabolic adjustments to sustain permissive soluble carbohydrates (SC) concentrations through temperature changes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

To run the model, a variant of a closed-loop control system that aims to maintain constant soluble carbohydrates concentrations in dormant trees was used. This model (Fig. 1a, b) incorporated the following biological assumptions:

- In temperate climates, with mild temperatures during winter (commonly ranging between 0 °C and 20 °C), stems in trees remains biologically active during dormancy.
- Stem parenchyma cells work to sustain SC within a range of SCm.
- Steady SC concentrations are only feasible if starch degradation activity (Kd) equals the sum of starch synthesis (Ks) and the respiration (R) rates.
- Starch synthesis is more sensitive to temperature changes than starch degradation ($Q_{10}s > Q_{10}d$) and their kinetic coefficients dictate a specific temperature of NSC equilibrium (Te) (Fig. 1a).
- Seasonal and diurnal temperature changes continuously challenge SC regulation as heat (T > Te) promotes starch synthesis and chill

(T < Te) induces SC accumulation (Fig. 1a).
- Winter respiration does not require all stored NSC, hence there is no substrate limitation and enzymatic activity is independent of substrate concentrations.
- High SC concentrations (SC > SCm) augment the starch synthesis frequency factor (As) by inducing the expression of its enzymes and pathways, while low SC concentrations (SC < SCm) augment the degradation frequency factor (Ad).
- The natural decay rate of proteins (λ), or their additional expression (ΔA), are constant and independent of temperature.

The C–T model (developed in an R environment) is iterative and computes SC in hourly steps (*n*) using temperature records to calculate starch synthesis (Ks), starch degradation (Kd), and respiration (R) rates (Fig. 1b, Code S1, and Fig. S2):

$$SC_{(n)} = SC_{(n-1)} + Kd(T_{(n)}, Ad_{(n-1)}) - Ks(T_{(n)}, As_{(n-1)}) - R(T_{(n)}) \quad (4)$$

As and Ad incorporate the size of the enzymatic group (i.e. amount of proteins), and they decay in every time step. Yet the C–T model increases As if SC exceeds SCm, and elevates Ad if SC falls short of SCm:

$$\mathrm{SC}_{(n)} > \mathrm{SCm}: \begin{cases} As_{(n)} = \lambda As_{(n-1)} + \Delta A \\ Ad_{(n)} = \lambda Ad_{(n-1)} \end{cases} \quad (5)$$

$$(SC_{(n)} < SCm)\ \&\ (ST > 0): \begin{cases} As_{(n)} = \lambda As_{(n-1)} \\ Ad_{(n)} = \lambda Ad_{(n-1)} + \Delta A \end{cases} \quad (6)$$

3.1.1. Deriving comparatives indices

Using classical terminology for comparative analysis (it isn't part of the model), the C–T model counts hours spent promoting starch synthesis as 'chill' accumulation, and hours spent promoting starch degradation as 'heat' accumulation, between senescence and bloom (n = N hours since senescence):

$$chill = \sum_{n=0}^{N} hour(SC > SCm) \quad (7)$$

$$heat = \sum_{n=0}^{N} hour(SC < SCm) \quad (8)$$

3.2. Initiation and parametrization

3.2.1. NSC concentration

NSC concentrations were analysed in almond twigs from the Central Valley, CA, during early winter (October through January 1st for 2016/17) to test if dormant trees regulate SC concentrations while they degrade starch. To initiate the C–T model, SCm (assumed to equal SC at senescence) was set to 85 mg g^{-1} DW (according to the almond samples collected at the 1st week of October). Subsequent sensitivity analysis exhibited that SCm of 85 mg g^{-1} DW was reasonable, located at the lower curvature of RMSE, but that reducing it to 79 mg g^{-1} DW would improve the model's fit to field observations (Fig. S5). Additionally, using data from January 15th through February 1st 2017 (24 samples) the threshold concentration for SC to trigger bloom (SCb) was estimated as 55 ± 16 mg g^{-1} DW (only past 100 DpS, as almond trees are unlikely to naturally bloom before January in both Israel and California).

3.2.2. Respiration (R)

Respiration was measured in the laboratory for almond twigs of various sizes, and the total NSC uptake for 100 days of winter was computed by the approach and parameters established in former studies (Sperling et al., 2015):

$$R_{(n)} = 0.00324 \bullet \exp[0.07 \times T_{(n)}] \quad (9)$$

3.2.3. Initial frequency factors ($As_{(0)}$ and $Ad_{(0)}$)

Summer turnover of starch in almond twigs was ~20 mg g^{-1} day^{-1} (preliminary studies) and we considered that it is likely to be much lower in winter. Hence, with no empirical data, we assumed that it could be lower than 10 mg g^{-1} day^{-1} if trees are dormant and set the initial $Ad_{(0)}$ to 0.1 mg g^{-1} h^{-1}, i.e. starch degradation rate (Kd) equals 0.5 mg g^{-1} h^{-1} at 25 °C. Preliminary computations exhibited that the initial Te should equal the average temperatures for the first week of October plus 3 °C to account for the exponential nature of the activity curves. Then, by $Ad_{(0)}$ and this empirical Te, assuming that steady SC concentrations equal SCm at time of senescence, the initial frequency factor for starch synthesis ($As_{(0)}$) was computed:

$$As_{(0)} = Ad_{(0)} \bullet \exp[T_e(Bd - Bs)] \quad (10)$$

3.2.4. Decay fraction (λ) and additional expression (ΔA)

Winter 2016/17 (from October 1st until January 10th) was used as

Table 1
Parameters and model constants.

Variables	value	Unit	Description
As		mg g^{-1} h^{-1} DW	Starch synthesis frequency factor
Ad		mg g^{-1} h^{-1} DW	Starch degradation frequency factor
$T_{(n)}$		°C	Hourly temperature
$SC_{(n)}$		mg g^{-1} DW	SC concentration
$ST_{(n)}$		mg g^{-1} DW	ST concentration
$Ks_{(n)}$		mg g^{-1} h^{-1} DW	Starch synthesis activity
$Kd_{(n)}$		mg g^{-1} h^{-1} DW	Starch degradation activity
$R_{(n)}$		mg g^{-1} h^{-1} DW	Respiration rate
DpS			Days post Senescence
Initial values			
$Ad_{(0)}$	0.1	mg g^{-1} h^{-1} DW	Starch degradation frequency factor
$SC_{(0)}$	85	mg g^{-1} DW	SC concentration
$ST_{(0)}$	85	mg g^{-1} DW	ST concentration
Constants			
SCm	85	mg g^{-1} DW	Metabolic threshold for NSC management
ΔA	25*10^−6	mg g^{-1} h^{-1} DW	Dose of increase to frequency factor (*de novo* expression)
SCb	55	mg g^{-1} DW	SC concentration that triggers bloom
$Q_{10}d$	1.8	–	Starch degradation temperature coefficient
$Q_{10}s$	3	–	Starch synthesis temperature coefficient
λ	1*10^−4	–	Decay fraction
Ar	0.00324	mg h^{-1} g^{-1} DW	Respiration frequency factor
Br	0.07	–	Energy of starch degradation activation

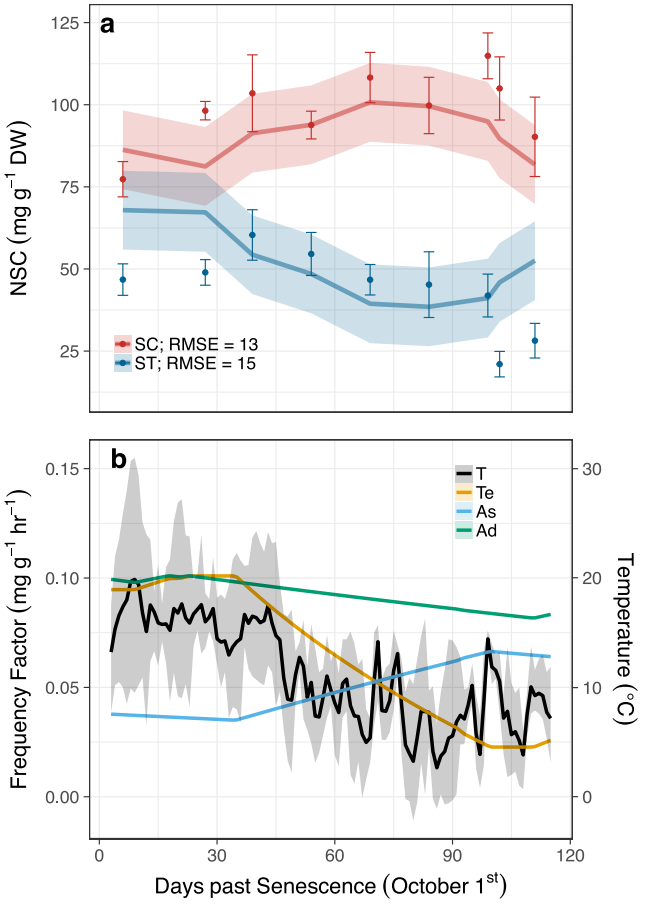

Fig. 2. Preliminary computations and field measurements to parameterize the C–T model. (a) Average field concentrations of soluble carbohydrates (SC, red dots ± se) and starch (ST, blue dots ± se) in almond trees for October 1st through January 30th (120 DpS) 2017. The samples were collected by the Carbohydrate Observatory (https://psfaculty.plantsciences.ucdavis.edu/plantsciences_faculty/zwieniecki/CR/cr.html) throughout the Central Valley of California during the 2016/17 winter and aggregated for every 3 consecutive days. Concurrently, the C–T model was parameterized to project compatible SC (red line) and root mean square error (RMSE = 13 mg g^{-1} day^{-1}, red ribbon) or ST (blue line and ribbon, RMSE = 15 mg g^{-1} day^{-1}) concentrations by the temperature recorded in Merced, CA. **(b)** Field temperatures (daily average for the 2016/17 winter as black line, ribbon denotes daily min and max), the C–T model's frequency factors for starch synthesis (As, blue line), starch degradation (Ad, green line), and the temperature of carbohydrate equilibrium (Te, orange line) for 120 DpS.

a training episode for the model, and the additional hourly increase in frequency factors (ΔA) or decay fraction (λ) for both pathways were set to meet the observed changes in SC and ST concentrations for this time. All model parameters and initial values are provided in Table 1.

4. Results

4.1. Model function

The Californian Carbohydrate Observatory for 2016/17 winter demonstrated that throughout the Central Valley, on average, almond twigs had 77 ± 5 mg SC g^{-1} DW and 47 ± 5 mg ST g^{-1} DW at the beginning of October (Fig. 2a). SC later peaked at 114 ± 7 mg SC g^{-1} DW in January 7 (99 DpS) while starch dropped to 42 ± 6 mg g^{-1} DW. The C–T model, that constantly adjusted the parameters of NSC metabolism to the hourly temperatures, projected corresponding NSC changes with a root mean square error (RMSE) of 12 mg g^{-1} DW for SC and 15 mg g^{-1} DW for starch to the measured NSC concentrations for 120 DpS. Alternatively, enzymatic computations with constants for NSC metabolism throughout dormancy, suggested (unrealistically) that 85 mg g^{-1} DW of ST should have solubilized within 75 DpS due to the cold and that respiration could only account for 27% of these NSCs (i.e. 22 mg g^{-1} DW, Fig. S3). To adjust NSC concentrations, the C–T model considered that, generally, between senescence and 80 DpS, trees were colder than Te, i.e. prone to hydrolyse starch, and they induced As to avoid excessive SC concentrations (and consequently ST depletion). Therefore, the C–T model implies that for the first 27 DpS, while temperatures change mildly, SC and ST would fluctuate about the initial SCm (85 ± 10 mg g^{-1} DW), independent of the arbitrary starting date (October 1st) we set. Then, as the environment cools, SC should increase and peak at 105 mg g^{-1} DW, while ST plunges to 50 mg g^{-1} DW, between 50 and 75 DpS (which is consistent with the field observations). At this point the C–T model projected a change in NSC management, where increased starch synthase activity promotes ST accumulation, and that by 100 DpS SC returns to 85 mg g^{-1} DW, while ST increases to 65 mg g^{-1} DW (accounting for a 20 mg g^{-1} DW loss to respiration).

On the enzymatic level, we initiated the C–T model assuming SC = SCm at 1st of October, when the daily temperature averaged 18.5 °C for the past 25 years, with $Ad_{(0)}$ of 0.1 mg g^{-1} DW h^{-1} and computed $As_{(0)}$ to be 0.024 mg g^{-1} DW h^{-1}. Interestingly, this showed that the initial temperature of NSC equilibrium (Te) was 21 °C, i.e. warmer than the daily average, due to the midday heat that exceeded 25 °C and exponential nature of the temperature kinetic curves. The C–T model then continued to compute NSC concenrations by a 0.01% hourly decay of As and Ad, and an increase to As or Ad (subject to SC concentrations) by 25*10^{-6} mg g^{-1} DW h^{-1} (ΔA, Fig. 2b). As the environment remained warm the first 28 DpS (most of October), sugar concentrations were within 10 mg g^{-1} DW range of SCm, and the model did not increase Ad or As (independent of the starting date once more). In fact, the C–T model projected Ad and As would decrease moderately at this time due to natural decay. Then, as the environment cooled from 30 DpS on, Ad continued to slowly decline from 0.093 mg g^{-1} DW h^{-1} to 0.078 mg g^{-1} DW h^{-1}, while As increased from 0.033 mg g^{-1} DW h^{-1} to 0.061 mg g^{-1} DW h^{-1} (200%), by 96 DpS (Fig. 2b). Te essentially mirrored the changes in As, being stagnant at 21 °C for the first 28 DpS, then steadily cooling to 5 °C by 96 DpS.

4.2. Carbohydrate concentrations projections

The C–T model was used to project winter SC concentrations in almond twigs for 25 years at Durham, Davis, Manteca, and Shafter (years 1985, 1990, 1995, 2000, 2005, and 2018 are specifically illustrated in Fig. 3a). These calculations indicate that SC concentrations in almond twigs would not increase, and often decrease, for the first 25 DpS. Then, the C–T model projected that SC concentrations increase for 50 days before they stabilize (peaking at 130 mg g^{-1} DW at 77 DpS in 2000). By ~100 DpS, daytime temperatures begin to rise, and the model projected an increase in As frequency, essentially increasing starch synthase and lowering SC levels again. Subject to the multi-annual temperature variability, SC concentrations would return to the original 85 mg g^{-1} between 100 and 120 DpS. The C–T model projected that SC concentrations would continue to drop due to the typical late winter warming in the Central Valley and reach SCb (55 mg g^{-1} DW), a threshold that would trigger bud– burst, by the approximated time of bloom (~140 DpS). Yet the exact day that SC concentrations would fall below 55 mg g^{-1} DW varied between 129 and 150 DpS because it strongly depended on the specific temperature trends of each winter.

Using all winter temperature data for four research sites in California, the C–T model considered 100 independent events of SC reaching SCb. It demonstrated that SC concentrations are sensitive to both chill hours (i.e. times trees induce As to avoid SC accumulation in the cold) and heat hours (i.e. times SC < SCm, Fig. 3b). Typically, As increased (i.e. chill) for 1550 ± 462 h and Ad (heat) for 1730 ± 490 h

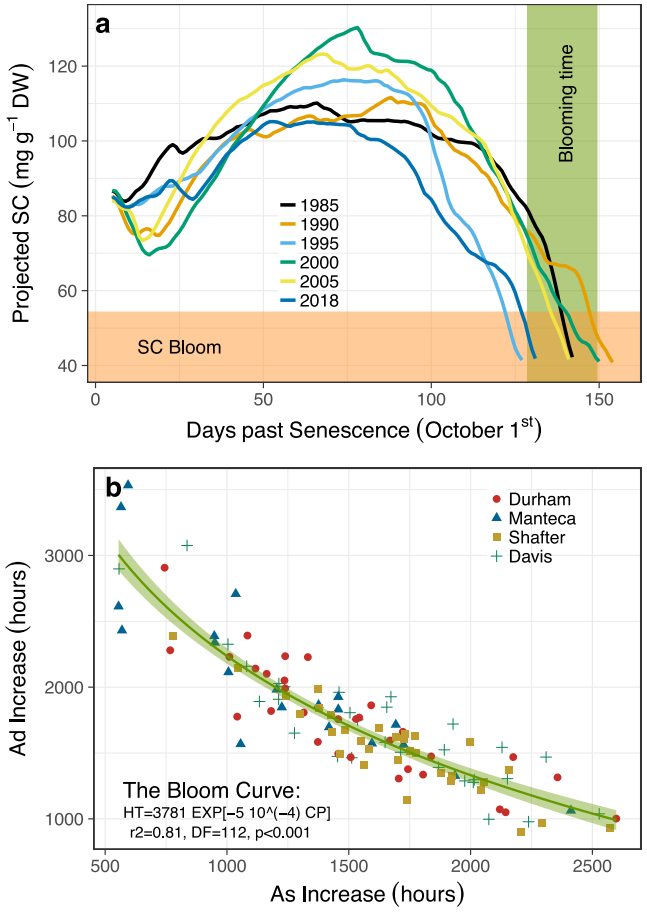

Fig. 3. Multi-annual C–T model projections. (a) The C–T model projections of soluble carbohydrate (SC) concentrations in Davis, CA, using temperature data for 155 DpS. It exhibited an increase for ~75 days past senescence (DpS), and then decrease to critical concentrations that could trigger bloom (orange region) at the approximate time of almond bloom (green region) for a series of years between the years 1985 and 2018. **(b)** The C–T model projections, processing historical temperature data (1983–2018) in Durham (red circles), Manteca (blue triangles), Shafter (orange squares), and Davis (blue crosses), for the time almond trees spent inducing their starch synthase frequency factor (As, 500–2,500 h) and its inverse exponential proportions ($r^2 = 0.81$, DF = 112, $p < 0.001$) to the time they spent inducing starch degradation (Ad, 1,000 and 3,000 h).

before SC reached SCb. Thus, the C–T projected average bloom date of 137 ± 10 DpS. Yet the C–T model also projected a non-linear, inverse, relationship between chill and heat times, such that warmer winters would delay bloom until 141 DpS while colder winters would delay it further until 149 DpS.

4.3. Bloom projections

The C–T model projected that SC would drop if trees are acclimated to synthesize starch, instead of accumulating SC in the cold, as days become warmer at the end of winter (typically after 100 DpS). Although respiration rises with increasing temperatures, it would consume a small portion of the missing SC, and this SC drop implies that ST would accumulate in deciduous species at the end of winter. Accordingly, almonds in California, almonds in Israel, peach in Israel, and pistachios in California induced their ST:SC ratio over 0.8 in Californian almonds, and up to 1.3 in Israeli almonds, by 125, 122, 100, and 160 DpS, respectively (Fig. 4a). This actually corresponded to the species and region relative blooming times, i.e. peach flowers first, then almonds, and finally pistachios, while almonds flower sooner in Israel than in California. Moreover, the Carbohydrate Observatory confirmed that in the winter of 2016/17 starch surged in almond trees throughout the Central Valley of California for 10 days between 120 and 130 DpS and preceded bloom time by ~10 days (Fig. 4b). Finally, the C–T model projections when SC concentrations in almond trees would drop to 55 mg g^{-1} DW correlated to the actual blooming times at Durham (years 1984–2008), Shafter (1996–2008), and Manteca (1996–2008) by a near 1:1 ratio with an RMSE of ~4.7 days (Fig. 4c). Importantly, the actual bloom times ranged between 126 and 150 DpS and averaged 139 ± 5 DpS, which supports our determination that SCb of 55 mg g^{-1} DW is valid for use in the C–T model for almond trees (later than 100 DpS).

5. Discussion

The C–T model attributes temperature induced changes in starch synthesis and degradation pathways to a strategy of trees to control their SC concentrations throughout winter. We hypothesized that such fundamental changes to NSC metabolism would also enable trees to gauge winter progression. Generally, temperature kinetics promote starch degradation as winter gets colder. Therefore, and somewhat counterintuitively, trees need to avoid excessive SC concentrations by promoting starch synthesis in winter. Yet, as starch synthesis is more sensitive to temperature changes than starch degradation, the C–T model suggested that warmer days at the end of winter would lead to abrupt SC losses and trigger bloom. Therefore, the C–T model integrates notions of 'chill' and 'heat' episodes in winter to a holistic perspective of winter temperature requirements of deciduous trees. Yet, the C–T model should be developed further to account for – 1) substrate concentrations (both ST and SC) in computing the activity rates (essentially making them rate constants), and 2) for temperature in setting the expression and decay functions to the metabolic activities.

The C–T model demonstrated that **adjusting the frequency factors in NSC metabolism could model how deciduous trees regulate SC concentrations in winter**. The diurnal temperature variations in winter challenge the delicate balance between SC and ST, and plants need to adjust the relevant metabolic pathways (possibly expression and decay of enzymatic proteins) to sustain permissive SC concentrations. In fact, with metabolism running low (respiration is minimized by the cold and growth is suppressed by dormancy), and temperature kinetics that promote starch degradation in the cold, the C–T model suggests that trees often need to promote starch synthesis in winter. This doesn't imply that trees don't need SC in winter. Yet it emphasizes that trees work to counterbalance temperature kinetics in winter, which dictate excessive starch degradation in the cold. Concurrently, as previously reported for multiple tree crops [e.g. cherries (Bandurska et al., 2009) and olives (Bustan et al., 2011)], the almond trees we studied increased SC concentrations modestly despite a major drop in average daily temperatures in the first 100 days of winter. They actually matched starch utilization to their respirational demands which could be attributed to winter adjustments of the starch synthesis and degrading pathways (Witt and Sauter, 1994; Yoshioka et al., 1988) that are triggered by SC concentrations (Koch, 2004).

The enzymatic activity approach of the C–T model could provide a new and mechanistic perspective to studies and modelling of winter physiology (Charrier et al., 2018). The model illustrates tight NSC regulation in winter, and attributes changes in temperature kinetics to the frequency factors of the starch synthesis and degradation pathways (As and Ad; Eqs. (4)–(6)). The C–T model considers that if SC shifts away from the permissive range, increasing the amount of enzymes would increase their frequency of collisions with the substrate in a liquid media (Arrhenius, 1889) and provide the necessary feedback regulation. Currently, the C–T model considers an empirical level for SCm (85 mg g^{-1} DW) which applied to all the tree species (almond, peach, and pistachio) and regions (California and Israel) we considered. Yet SCm could significantly change for trees in colder climates that

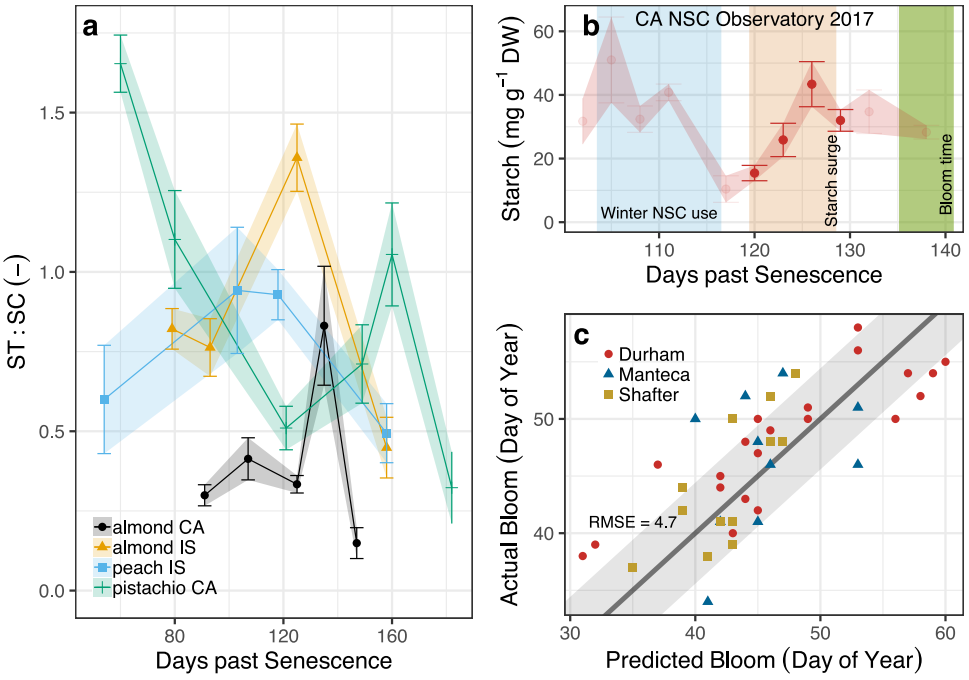

Fig. 4. Bloom C–T model projections. (a) The ratio between starch and soluble carbohydrates (ST:SC) in peach trees in Israel (blue line denotes average values and error bars and ribbons represent ± se), almond trees in Israel (orange), almond trees in California (black), and pistachio trees in California (green). **(b)** Starch concentrations in almond orchards throughout the Central Valley of California at the time they support winter metabolism (blue region), as they surge prior to bloom (orange region, bold symbols), and as they stabilize at bloom time (green region) during the winter of 2016/17. **(c)** The C–T model projections of bloom time by SC reaching the 55 mg g^{-1} DW threshold versus the actual bloom records from Durham (1984–2008, red circles), Manteca (1996–2008, blue triangles), and Shafter (1996–2008, orange squares). The 1:1 ratio is denoted by a black line and the root mean square error (RMSE = 4.7 days) by a grey ribbon.

should sustain higher levels of available energy and osmolites to withstand winter (Améglio et al., 2001). Alternatively, SCm might be lower for trees in milder environments that risk early depletion of their winter carbohydrate reservoirs (Sperling et al., 2015). Adjustments to the frequency factors would also change the temperature of NSC equilibrium (Te) and integrate the 'chill' and 'heat' concepts to account for all the thermal conditions in winter. Since Te changes according to the enzymatic adjustments that should keep SC at SCm, the C–T model associates chill with 16 °C in November, and with 6 °C (a common threshold in past models) only in February. Forcing temperatures (heat) on the other hand could be as low as 7 °C in mid-winter and rise to 10 °C in spring. Consequently, with no fixed temperature thresholds, the C–T model could fit a wide range of climates. Yet further studies are needed to establish key properties of the C–T model. For now, we assumed that the activation energy of specific pathways (Bs or Bd) don't change, as they are fundamental properties of enzymes (Gupta and Kaur, 2005). Yet we should revisit this option in the future and extend our studies to the analysis of catalytic activities, and potentially gene expression, of the relevant enzymatic groups. Secondly, we considered the natural decay and expression rates independent of temperatures, and yet linking them to temperature changes (presumably following the Arrhenius concept) could make the C–T model suitable for colder environments where metabolic activity is much lower in winter. Finally, we need to thoroughly examine the model's implication that at the end of winter trees might excessively synthesize starch and abruptly drop SC concentrations.

NSC concentrations in almond peach, and pistachio trees in California and Israel supported the C–T model projection that **winter adjustments of NSC metabolism lead to a metabolic deficiency that precedes bloom.** Generally, starch degradation should recharge SC concentrations to suffice for respirational demands in fall and early winter (Loescher et al., 1990), and flowering at time of anthesis (Bustan and Goldschmidt, 1998). Consequently, SC concentrations should be stable in winter, and if they increase due to acute stress [e.g. frost (Améglio et al., 2001; Livingston and Henson, 1998)] or decrease due to depleted storage, it could compromise bloom. Yet the C–T model suggests that once daytime temperatures rise in the spring, a critical loss of SC in late winter is inevitable due to elevated respiration, and promotion of the starch synthesis pathway. Accordingly, the model projects that starch content would surge in the spring, which is consistent with our former report (Sperling et al., 2017b), evident in figures of multiple publications (Ito et al., 2012; Kaufmann and Blanke, 2017; Sauter and van Cleve, 1994), and has been associated with chilling and bloom in cherry trees (Fadón et al., 2018).

This starch surge measurement, which is rarely discussed in the literature where we recognized it (Ito et al., 2012; Kaufmann and Blanke, 2017; Sauter and van Cleve, 1994), evidently supports the mechanism we propose in the C–T model. It emphasizes that, due to the exponential nature of Ks, a few hot hours during midday following the cold winter would have a bigger effect on starch accumulation than the long cold nights on starch degradation. Additionally, prolonged periods of as increase (i.e. 'chill' hours) would amplify starch synthesis and critical SC losses could occur after a shorter warm period (i.e. less 'heat'). Alternatively, in warmer winters NSC metabolism may not have acclimated to the cold, and SC concentrations will require more time in warmer conditions, or much warmer days, to drop. This supports the notion of coupling the chill and heat approaches to forecast bloom time (Chuine, 2000; Harrington and Gould, 2010; Pope et al., 2014). In fact, if SC is low, an energetic deficiency could trigger bloom, as does water stress (Southwick and Davenport, 1986) and mechanical stresses (Sanyal and Bangerth, 1998). Low SC concentrations would be a very strong ecological signal for trees to induce reproduction if the prospect their survival is jeopardized. Likewise, traditional farming practices that induce bloom seem to target winter NSC metabolism, often unknowingly, in tree crops. For instance, winter sprays of chill substitutes (e.g. hydrogen cyanamide) that temporarily inhibit respiration and 'starve' the reproductive branches. Such short-term metabolic deficiency is a novel physiological link between the chill and the forcing-temperatures concepts. Additionally, the SC drop, or the starch surge, could be applied as a physiological indicator to bloom management, which becomes necessary due to growing variability in winter conditions (Campoy et al., 2011).

Corroborating the C–T model with historical data sets for almond blooming times in California demonstrated that **NSC status could indicate if winter temperatures are favorable to deciduous trees and when they will bloom.** Specifically, the low SC concentrations and the starch surge could act as a pre-indication to bloom. Evidently, starch surged in peach first, then in almonds in Israel, later in almonds in California, and finally in pistachios in California, which was consistent with their blooming times. The C–T model does not attribute chill and

heat to different parts of winter to forecast bloom, but integrates all the winter temperature records, high and low, between autumn senescence and spring. In this sense the C–T model follows up on the Dynamic Model (Erez et al., 1990), while incorporating a physiological framework, and extending its output to forecasting bloom. Conveniently, the C–T model isn't affected by the arbitrary starting date of October 1st, as it doesn't exhibit metabolic adjustments until temperatures begin to actually decline in December. Importantly, the C–T model predicts that trees will bloom every year, even if winters are warm and the empirical models don't score enough 'chill'. The C–T model does predict that abnormal winter conditions could extend dormancy by up to two weeks, similar to what has been reported (Pope et al., 2014) as blooming times range between 137 DpS in average years, 142 DpS in warmer years, and 151 DpS and cold years.

The pre-bloom SC deficiency should be reversed to guarantee the energetic demands of vital flowers by activation of NSC transport between roots and the branches carrying reproductive buds (Loescher et al., 1990). A pre-condition of SC transport could explain why the effectiveness of winter farming applications to break dormancy, e.g. pruning and oil sprays, require sufficient chill (Erez, 1995). The C–T model also demonstrates that for each chill level, heat can still vary by ˜150 h (nearly a week) before the trees will bloom. This implies that there might be additional attributes to blooming beyond temperatures, or that a closer look at temperature dynamics and rates of changes is needed. Specifically, as was suggested previously, not all chill (or heat for that matter) should be treated equal (Asse et al., 2018; Luedeling et al., 2013). According to the C–T model, if temperatures drop early in winter trees significantly update their starch synthase capacities to sustain permissive SC concentrations at low temperatures. Then, periodic heat episodes in early February would critically affect SC and synchronize bloom across many trees. This would attract and sustain large populations of pollinators (Bartomeus et al., 2013), maximize cross-pollination (Méndez and Díaz, 2001), and induce reproduction. Alternative winters though, which begin warm and cool only later, will require more heat to trigger breaking of dormancy and explain intra-species variability in bloom time. Evidently, the C–T model isn't confined to the attributes of semi-arid environments. In fact, with additional ground-work to determine seasonal metabolic levels, it could extend to other tree species. The immediate matters would be field indications to guide artificial dormancy breaking [a common practice in stone fruits (Erez, 2000)] and yield forecasts for crops that require expensive processing (e.g., nuts, citrus, and vines (Luedeling, 2012b)). Yet, in the long run, the C–T model would benefit the industry more if farmers use it to appraise the fit of deciduous tree crops to new environments, and nurseries reassess their breeding programs by it. In addition, ecologists could use the C–T model to estimate the risk of NSC depletion in natural populations due to climate shifts (Dunn et al., 1987).

6. Conclusions

The C–T model and NSC field observations provide a mechanistic and physiological link between chill requirements, the forcing-units concepts, and bloom forecasts. According to the C–T model cold is essential for vital blooming, and yet it is heat that causes a metabolic deficiency that triggers trees to bloom. Practically, a detectable starch surge precedes blooming by over a week, low SC concentrations correlate to bloom, and the C–T model's past projections of bloom time fit the reports. Additionally, the C–T model is insensitive to its arbitrary starting time as it only responds to winter's prominent temperature changes that would alter metabolism. Yet the model needs further development, concerning the annual level of metabolic adjustments, time and magnitude of the starch surge, and the rate of SC uptake following the metabolic shift to starch synthesis, to effectively forecast bloom time.

Author contribution

OS and MAZ led the research and formulated the C–T model. TK and ER tested the model and worked the details to its final formalization. AD analyzed carbohydrates and parameterized the model by the seasonal trends. AT established the temperature kinetic aspect of the C–T model. KJS and TD studied almond winter phenology and validated the C–T model. Finally, OS, TK, AT, AD, KJS, ER, TD, and MAZ illustrated the results and wrote the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agrformet.2019.107643.

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